A Section 508–conformant HTML version of this article is available at https://doi.org/10.1289/EHP635.

Weekly Personal Ozone Exposure and Respiratory Health in a Panel of Greek Schoolchildren

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Background: The association of ozone exposure with respiratory outcomes has been investigated in epidemiologic studies mainly including asthmatic children. The findings reported had methodological gaps and inconsistencies.

Objectives: We aimed to investigate effects of personal ozone exposure on various respiratory outcomes in school-age children generally representative of the population during their normal activities.

Methods: We conducted a panel study in a representative sample of school-age children in the two major cities of Greece, Athens and Thessaloniki. We followed 188, 10- to 11-y-old, elementary school students for 5 wk spread throughout the 2013–2014 academic year, during which ozone was measured using personal samplers. At the end of each study week, spirometry was performed by trained physicians, and the fractional concentration of nitric oxide in exhaled air (F_{eNO}) was measured. Students kept a daily time–activity–symptom diary and measured PEF (peak expiratory flow) using peak flow meters. Mixed models accounting for repeated measurements were applied.

Results: An increase of 10 $\mu g/m^3$ in weekly ozone concentration was associated with a decrease in FVC (forced vital capacity) and FEV_1 (forced expiratory volume in 1 s) of 0.03 L [95% confidence interval (CI): −0.05, −0.01] and 0.01 L (95% CI: −0.03, 0.003) respectively. The same increase in exposure was associated with a 11.10% (95% CI: 4.23, 18.43) increase in F_{eNO} and 19% (95% CI: −0.53, 42.75) increase in days with any symptom. The effect estimates were robust to PM_{10} adjustment. No inverse association was found between ozone exposure and PEF.

Conclusions: The study provides evidence that airway inflammation and the frequency of respiratory symptoms increase, whereas lung function decreases with increased ozone exposure in schoolchildren. https://doi.org/10.1289/EHP635

Introduction

Ozone (O_3), a very reactive gas and strong oxidant, is found as a secondary pollutant in the troposphere. Although, its presence in the stratosphere is essential for life because it filters harmful ultraviolet radiation, increased concentrations of O_3 in the air we breathe have been linked to adverse health effects mainly concerning the respiratory system (WHO 2013). A number of controlled human exposure studies have demonstrated changes in FEV_1 and respiratory symptoms (Adams 2006; Horstman et al. 1990; Kim et al. 2011) as well as lung inflammation (Devlin et al. 1991; Kim et al. 2011), but those included healthy adults, not children. In an attempt to investigate the acute effects of ozone under real-world exposures several camp studies have been conducted involving sequential measurements of lung function and ambient ozone measurements on children attending summer camps (Burnett et al.1990; Higgins et al. 1990; Raizenne et al.1989; Spektor et al. 1988; Spektor et al. 1991). However, despite the evidence they provide of an association between daily outdoor O_3 concentrations and decreased lung function (Kinney et al. 1996), the lack of personal measurements, variations in data reporting and analysis as well as concerns about potential confounding by other pollutants that may co-vary with O_3, limit their use in risk assessment. Therefore, so far there is lack of studies conducted in children under real living conditions, at current concentrations of ozone, using personal exposure assessment.

Children's lungs are considered to be more sensitive and vulnerable to air pollution as they have, even at rest, a larger surface area for body size compared with adults and are still growing. Children breathe more air per unit of body weight and have different breathing patterns than adults that alter the deposition and toxicity of inhaled gases and particles (Arcus-Arth and Blaisdell 2007; Bateson and Schwartz 2008; Warren et al. 1990). Moreover, because they spend more time outdoors and are more active, they have higher ventilation rates leading to increased intake and deposition of pollutants (Bateson and Schwartz 2008). So far, epidemiological studies on the effects of ozone on children's lung function and respiratory symptoms have mainly focused on asthmatics and have reported inconsistent results (Castro et al. 2009; Declercq and Macquet 2000; Jalaludin et al. 2000; Just et al. 2002; Li et al. 2012; Mortimer et al. 2002; Scarlett et al. 1996). However, response to ozone exposure displays individual variability that cannot be explained by underlying morbidity (Schelegle et al. 2012; WHO 2013), and thus, from a public health point of view, it is critical to determine to what extent ambient O_3 exposure is responsible for adverse respiratory effects in the general population of children.

We conducted a panel study (Respiratory Effects of Ozone Exposure in children; RESPOZE) in a representative sample of the general population of schoolchildren in the two major cities of Greece, Athens (state capital) and Thessaloniki. Greece provides a unique opportunity to study O_3 health effects because of its sunny, warm weather as well as the high concentrations of precursor pollutant emissions encountered in some of its cities.

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The authors declare they have no actual or potential competing financial interests.

Received 9 June 2016; Revised 8 March 2017; Accepted 13 March 2017; Published 21 July 2017.

In this paper we examine the association of weekly personal ozone measurements with lung function parameters, airway inflammation, and respiratory symptoms.

Materials and Methods

Study Design: Subjects

A panel study was conducted, in the two largest and most polluted cities of Greece, Athens and Thessaloniki (total population of about 4 million). The research was approved by the Ethics Committee of the National and Kapodistrian University of Athens, and it complied with all relevant national and local regulations. The study population consisted of all children in the fifth grade (age 10–11 y) in state elementary schools representative of the general population of students in that age group. State schools were chosen because all attending students are required to live in the school neighborhood. A two-stage sampling design was applied. First, state elementary schools located near a fixed monitoring site (within 2 km), were identified, and permission to implement the project was obtained from the Ministry of Education. In the second stage, the research team visited all schools and informed fifth grade school children in class about the project. Informed consent was obtained from the parents of children willing to participate and the final sample was formed. We had estimated that a sample of 200 school children (100 from Athens and 100 from Thessaloniki) would provide power >90% to detect a decrease in FEV_1 (forced expiratory volume in 1 s) of 0.14% per 10 $\mu g/m^3$ in O_3 exposure, at the 5% significance level, considering that the overall proportion of variance explained would be 41%. In each city 60% of the sample was drawn from high-O_3 areas (suburbs), to ensure that we had enough relatively highly exposed children, and 40% from low-O_3 areas (city centers, where ozone is scavenged by primary pollutants). All field work was implemented during the academic year 2013–2014 and consisted of five weeks of intensive follow-up per study participant: two in the fall period, one in winter, two in spring/summer. During each period, we used a staged entry of the participants for practical reasons. As a result, the period of data collection was longer, and thus, the likelihood for uncontrolled factors or unexpected events to influence the association between air pollution and health decreased (Roemer et al. 2000). Before the start of the field work, trained interviewers visited the schoolchildren's families at home to fill in an extensive questionnaire with information on demographic, life style, and residential characteristics and the medical history of the child. During this visit they also provided a peak flow meter (Mini-Wright, Clement Clarke International Ltd.) and trained the student on how to use it. A team of three field workers, including one pediatrician or pulmonologist, visited each school twice for each field work week. At the first visit an O_3 personal sampler (Ogawa USA) and a time–activity diary (TAD) were given to the participating students. At the second visit, a week later (same weekday), the O_3 personal sampler and completed TADS were collected, spirometry was performed, the fractional concentration of nitric oxide in exhaled air (F_{eNO}) was measured and a 24-h dietary recall questionnaire was filled in by interview. [See "TAD-PEF (Peak Expiratory Flow): Symptoms"; "Spirometry"; "FeNO"; "Ozone Exposure Assessment"].

TAD-PEF (Peak Expiratory Flow): Symptoms

Participating schoolchildren were instructed to complete a TAD collecting information on his or her location (at home, outdoors, indoors, but not at home and in transport) at 15-min intervals for each day of the field work period. In the TAD, they additionally recorded a total of nine PEF recordings per day, performed with the Mini-Wright peak flow meter three times every morning, afternoon, and night before the use of any medication. In addition, at the end of each day they were asked to record all their daily symptoms, namely cough, wheezing, breathing difficulties (dyspnea), fever, and stuffy nose; school absenteeism; and daily medication use (yes vs. no, as well as identification of medicine).

Spirometry

Spirometry was performed at school at the end of each of the five intensive field work weeks. Thus, a total of five spirometric assessments were available per schoolchild. All spirometry maneuvers were performed by the same pulmonologists or by the specially trained pediatrician in a separate office provided by each school that was quiet, well ventilated and had a standard temperature independent of season. Children were tested in a seated position and with nose clips in place. Portable spirometers (Spiropalm, Cosmed Srl, Italy) compatible with ATS (American Thoracic Society) and ERS (European Respiratory Society) requirements were used (Miller et al. 2005). At least two technically satisfactory maneuvers had to be performed with a difference between the two highest values of FVC (forced vital capacity) and FEV_1 of <5% of each other. The best FVC and FEV_1 were recorded independently of the order of the maneuver, whereas the values for the other parameters were chosen from the maneuver having the largest sum of FVC and FEV_1 (Miller et al. 2005). PEF and $FEF_{25–75}$ were also recorded.

F_{eNO}

A measurement of F_{eNO} was performed at school at the end of each field study week just before spirometry in the same room where spirometry was performed (see above), thus, a total of five measurements were available per schoolchild. The NIOX MINO (Aerocrine, Solna, Sweden), an instrument that has also a scrubber to eliminate ambient NO from the subject's sample, was used for all measurements according to American Thoracic Society (ATS) and European Respiratory Society (ERS) recommendations (ATS/ERS 2005).

Ozone Exposure Assessment

To assess O_3 weekly exposure, each student was provided with a personal Ogawa O_3 sampler (Ogawa & Co. USA Inc., Pompano Beach, FL) at the first visit of each field work week and was instructed on how to wear and handle it. The sampler was collected during the second visit at the end of the week. One sampler was placed in the outdoor space of each participating school also providing a weekly measurement. Additionally, concentrations of daily ambient O_3 as well as PM_{10} (particulate matter with aerodynamic diameter $\leq$10 μm) and NO_2 (nitrogen dioxide) were obtained by the nearest fixed monitoring site from the state network (http://www.ypeka.gr/).

Confounder Data

A possible learning or fatigue effect as well as a growth factor throughout the academic year was taken into account by including a time trend as a variable taking values 1–5 for each consecutive week (week ID). Additionally, all models were adjusted for sex, city (i.e., Thessaloniki vs. Athens) and area (high- vs. low-ozone area, as used in the sampling frame), father's education (years) as socioeconomic index, outdoor ambient temperature (°C, weekly average and alternatively same day temperature), consumption of antioxidant foods (yes/no, in the corresponding 24-h recall), time spent outdoors (hours/day, from data recorded in the corresponding week TAD), and medication use (yes/no,

during the corresponding week). Height (cm) and weight (kg) were adjusted in models with lung function outcomes. Models were additionally adjusted for PM_{10} levels from the nearest fixed monitoring site.

Quality Assurance and Quality Control

Standard operating procedures (SOPs) were applied for air pollution and health measurements. A training workshop for all field workers was organized before the start of the fieldwork. It should be noted that especially for the TAD, which are demanding on the subject's time and accuracy, scrupulous data quality procedures were applied as well as controls with manual inspections of the data and cross references from the field workers' progress diaries.

Statistical Analyses

Random effects models, incorporating a random intercept, Stata Statistical Software (Release 12; StataCorp LP), were used to account for the repeated measurements, for each child. We applied multiple linear regression models when we investigated O_3 effects on continuous variables: PEF measurements from the Mini-Wright (taken as the average of seven daily values calculated as the mean of the three maximum values from the morning, afternoon, and evening measurements), spirometry indices FVC, FEV_1, PEF, and $FEF_{25–75\%}$ (forced expiratory flow at 25–75%) as well as F_{eNO} measurements, which were log-transformed because the distribution was skewed. Poisson models were used for counts: the number of days per week when any symptom occurred and the number of days when the child was absent from school. In all models, we adjusted for all the potential confounders mentioned above. Because ozone concentrations are higher in spring–summer, we repeated the analysis for this period only. In addition, because asthmatic children may present special characteristics, we repeated the analysis excluding this subgroup using two alternative definitions. The first definition was based on parents' reporting a doctor-diagnosed asthma. The second definition further included those students having FEV_1/FVC and/or $FEF_{25-75\%} < LLN$ (lower limit of normal) at least once during the study period (provided that all spirometry maneuvers were performed according to the project's protocol), which provides evidence of obstructive ventilatory defect (Lougheed et al. 2012; Lum and Stocks 2010) and may denote (undiagnosed) asthma. We also conducted additional sensitivity analyses in healthy children only, excluding alternatively the two groups (asthmatic and asthmatic plus those presenting obstructive ventilatory defect) according to the definitions above.

Results are presented per 10-$\mu g/m^3$ increase in O_3 levels, representing approximately an interquartile range of the weekly measurements and thus, allowing comparability with previously published results based on measurements from fixed sites monitors. In sensitivity analyses we tested for heterogeneity between children, which included adding random slopes to our models.

Results

The final sample included 188 school-age children (97 in Athens and 91 in Thessaloniki). Table 1 shows personal, medical, socioeconomic characteristics, and respiratory health indices of the study population, by city and ozone concentration area (as used for the sampling scheme), during the academic year 2013–2014. Based on demographic data children residing in the two cities were similar. Parents reported a doctor-diagnosed asthma for 21 schoolchildren (7 in Athens and 14 in Thessaloniki). Of the remaining 167 children without a reported history of asthma, 22 (10 in Athens and 12 in Thessaloniki) had FEV_1/FVC and/or $FEF_{25-75\%} < LLN$ (lower limit of normal) for at least once during the study period.

In Table 2 descriptive characteristics of the air pollutants and temperature by city and high- or low- O_3 area (as used in the sampling scheme) are presented. O_3 personal measurements were much lower than outdoor measurements at schools or fixed sites, reflecting the amount of time spent indoors, where ozone concentrations are lower. Furthermore, O_3 as well as PM_{10} concentrations and temperature were higher in Athens than in Thessaloniki. Personal ozone and outdoor school ozone concentrations were in a range of 25–40% higher in high-versus low-ozone areas in both cities. In Athens, PM_{10} concentrations were lower by 20% in high- compared with low-ozone areas but, in contrast, in Thessaloniki they were 5% higher in high-ozone compared with low-ozone areas. Therefore, in Athens there was a larger contrast in both O_3 and PM_{10} concentrations between high- and low-ozone areas. In additional, contrasts were higher in outdoor school measurements compared with personal measurements.

Concerning the assessment of health effects, a negative association between weekly O_3 exposure and FVC was observed (both in the one and two pollutants models including PM_{10}). An increase of 10 $\mu g/m^3$ in weekly O_3 concentration was associated with a decrease in FVC of 0.03 L or 30 mL (95% CI: −0.05 L, −0.01 L). When we restricted the analyses to the spring–early summer period the negative effect of O_3 on FVC persisted. The results concerning the weekly O_3 effect on FEV_1 were similar. A 10-$\mu g/m^3$ increase in weekly O_3 concentration was associated with a decrease in FEV_1 of 0.01 L or 10 mL (95% CI: −0.03 L, 0.003 L). Peak expiratory flow, as assessed by spirometry, and $FEF_{25–75\%}$, were not associated with weekly O_3 exposure in any period of analyses (Table 3). Weekly averages from daily values of PEF, taken with the Mini-Right flow meter, were positively associated with O_3 and this association remained when we restricted the analyses to the spring–early summer period (Table 3). Inclusion of PM_{10} did not change the above-mentioned results.

Analysis of the association of F_{eNO} with O_3 showed that a 10-$\mu g/m^3$ increase in weekly personal O_3 concentration was associated with an increase in F_{eNO} values by 11.10% (95% CI: 4.23%, 18.43%). The direction of the effect and the magnitude of the association was practically the same after adjusting for PM_{10} [9.5% (95% CI: 2.46%, 16.98%)] (Table 3). Associations with F_{eNO} remained significant when we restricted the analysis in the spring–early summer study period 11.79% (95% CI: 2.54%, 21.82%).

An increase of 19% (95% CI: −0.53%, 42.75%), (21%, 95% CI: 0.42%, 45.66%, after adjusting for PM_{10}) in the number of days with at least one symptom within each of the five weeks was associated with a 10-$\mu g/m^3$ increase in weekly ozone exposure (Table 4). The association was smaller and nonsignificant when only the spring–summer period was considered (data not shown). No association between weekly O_3 personal exposure with "any absenteeism" (i.e., the number of days the student was absent from school during each of the five weeks) was observed (Table 4). In sensitivity analyses, the direction of the effects remained unchanged when the groups of asthmatic, asthmatic plus those having obstructive ventilatory defect, or healthy children were analyzed separately. We observed no significant heterogeneity in the effects between children when we allowed for random slopes in our models (data not shown). Results remained unchanged when the same day temperature was included in the models. In addition, no effect modification by area (high or low ozone) or city (Athens or Thessaloniki) was detected.

Discussion

In this panel study, we found consistent associations between increased weekly O_3 personal exposure and a decrease mainly in

Table 1. Personal, medical, socioeconomic characteristics, and respiratory health indices (presented as 5-wk averages) of the study population, by city and ozone concentration area.

	Athens		Thessaloniki	
	Ozone concentration area[a]			
Characteristic/respiratory health outcome	Low ($n=37$)	High ($n=60$)	Low ($n=33$)	High ($n=58$)
Boys [n (%)]	22 (59.5)	28 (46.7)	14 (42.4)	29 (50.0)
Age (y)	10.3 ± 0.3	10.3 ± 0.3	10.4 ± 0.4	10.4 ± 0.3
Height (cm)	147.2 ± 6.7	143.5 ± 7.7	145.9 ± 9.5	144.3 ± 7.3
BMI (kg/m^2)	18.2 ± 2.8	18.5 ± 3.6	18.0 ± 3.4	18.0 ± 2.8
Father's education (y)	14.0 ± 2.8	15.2 ± 3.7	15.3 ± 3.4	14.1 ± 3.3
Working father [yes; n (%)]	29 (78.4)	55 (91.7)	27 (81.8)	52 (89.7)
Asthma [n (%)]	2 (5.4)	5 (8.3)	4 (12.1)	10 (17.2)
FVC (L)	2.5 ± 0.4	2.4 ± 0.3	2.6 ± 0.4	2.5 ± 0.4
FEV_1 (L)	2.2 ± 0.4	2.1 ± 0.3	2.2 ± 0.4	2.2 ± 0.4
PEF (L/s)	4.7 ± 0.8	4.8 ± 0.8	4.9 ± 0.9	4.8 ± 0.9
$FEF_{25–75\%}$ (L/s)	2.6 ± 0.7	2.5 ± 0.5	2.6 ± 0.8	2.5 ± 0.6
F_{eNO} (ppb)	17.7 ± 17.8	15.6 ± 12.3	15.7 ± 13.8	16.5 ± 15.5
PEF-Mini-Wright[b] (L/min)	293.5 ± 53.9	287.8 ± 45.3	300.8 ± 55.8	297.5 ± 58.3
Students with any symptom during the study period[c] [n (%)]	29 (78.4)	40 (66.7)	27 (84.4)	45 (79.0)
Students with at least 1 d absence during the study period [n (%)]	18 (48.7)	31 (51.7)	12 (37.5)	22 (38.6)

Note: Data are presented as mean ± SD or n (%), unless otherwise stated.
[a]This definition of high- and low-concentration areas was based on previous years and used as a basis for the sampling procedure.
[b]Weekly averages from daily peak expiratory flow values (measured with the personal Mini-Wright peak flow meter) were used.
[c]At least one symptom during the study period.

FVC and also FEV_1, in 10-y-old school children following their normal daily schedules not modified by their participation in the study. We also observed corresponding increases in the number of days that any respiratory symptom occurred and in F_{eNO}, a marker of airway inflammation. Our sample included asthmatic children (about 7%), but separate analyses by asthmatic and non-asthmatic children did not show a modification in the effect estimates. The students were sampled from low- and high-O_3 areas and from two major cities, but no effect modification by area or city was detected.

Previous longitudinal studies investigating the effect of short-term exposure to O_3 on children's respiratory health involved mainly asthmatic children and relied on fixed monitoring sites rather than on personal measurements (Li et al. 2012). Most studies used PEF as lung function parameter. Moreover, among the few panel studies concerning healthy children no one, to the best of our knowledge, combined inflammatory response and change in lung function with personal measurements of O_3 exposure (Barraza-Villarreal et al. 2008; Chen et al. 2015).

One very consistent and robust finding in our study is the increase in F_{eNO} that persisted after controlling for PM_{10}, probably reflecting the capacity of O_3 to induce airway inflammation (Mudway and Kelly 2000) even in healthy children. Very few studies investigated exposure to O_3 and F_{eNO} levels. A panel study, involving school children living in Mexico City, demonstrated an association of F_{eNO} with acute exposure to traffic-related air pollutants in both asthmatics and nonasthmatics (Barraza-Villarreal et al. 2008). However, the 8-h moving average outdoor concentration of O_3 was associated with F_{eNO} in asthmatic children only. Another study investigated the effects of exposure to several pollutants on F_{eNO} in asthmatic children in the Mexican-U.S. border and found respiratory effects attributed to PM (several size fractions) and NO_2 but not of O_3 (Sarnat et al. 2012). Exposure assessment in these studies was based on fixed monitoring sites and passive samplers located in schools, resulting perhaps in less accurate assessment of O_3 exposure.

Furthermore, we found significant negative associations, robust after adjusting for potential confounders and more strongly during warmer days, between O_3 weekly personal exposure and FVC as well as FEV_1 (but of borderline significance).

Based on our results, the mean decrease found in FVC (0.0 3L, per 10-$\mu g/m^3$ increase in personal O_3 exposure) may be of clinical significance if repeated exposures lead to a more permanent adverse effect. Moreover, the range of personal exposure measurements is about 40 $\mu g/m^3$, depending not only on ambient concentrations but also on the child's time spent outdoors, and in the highest exposure range the weekly decrease in FVC may be clinically relevant. Concerning our findings on F_{eNO}, it follows that a 20% increase in F_{eNO} is associated with 20-$\mu g/m^3$ increase in O_3 personal exposure (an exposure contrast well within the range of measured exposures from 1 to 42 $\mu g/m^3$) considered to indicate an effect that may have clinical importance (Dweik et al. 2011).

An unexpected finding of our study was the significant positive associations of O_3 with the weekly averages of daily PEF self-assessed values using Mini-Wright peak flow meters. PEF is the most usual measurement in panel studies evaluating the effect of air pollution on children's lung function but results have been inconsistent across studies. Some panel studies reported PEF decrements in healthy (Declercq and Macquet 2000; Höppe et al. 2003; Li et al. 2012) and asthmatic school children (Just et al. 2002; Li et al. 2012; Mortimer et al. 2002; Romieu et al.1997) as well as enhanced daily PEF variability in the asthmatics (Just et al. 2002; Li et al. 2012), whereas others found no effect (Peacock et al. 2003; Scarlett et al. 1996). Likewise, in the older studies of children attending summer camps in the United States and Canada, inconsistent results associating ambient O_3 exposure

Table 2. Weekly air pollution and temperature levels by city.

	Athens		Thessaloniki	
	Ozone concentration area			
Environmental indicator	Low ($n=37$)	High ($n=60$)	Low ($n=32$)	High ($n=57$)
O_3 personal measurements ($\mu g/m^3$)	8.2 ± 6.7	10.8 ± 7.8	4.7 ± 4.8	5.9 ± 6.6
O_3 outdoor at schools ($\mu g/m^3$)	45.9 ± 14.7	64.3 ± 20.1	35.2 ± 20.7	45.6 ± 19.4
O_3 at fixed sites ($\mu g/m^3$)	24.6 ± 13.8	63.8 ± 16.4	36.3 ± 16.7	41.3 ± 18.5
PM_{10} at fixed sites[a] ($\mu g/m^3$)	28.9 ± 7.4	23.1 ± 7.1	18.9 ± 3.8	21.0 ± 9.3
Temperature (°C)	17.6 ± 2.5	17.6 ± 2.5	14.6 ± 3.9	13.7 ± 3.8

Note: Data are presented as mean ± SD. The high- or low-O_3 concentration areas were used for the sampling scheme.
[a]Average of 24-h values.

Table 3. Mean change in lung function indices associated with weekly increase of 10 μg/m^3 in O_3 exposure measured by personal monitors in 178 school children.

		O_3 effect per 10 μg/m^3			
		All study periods		Spring–early summer period	
Health outcome	Models	β-coefficient (95% CI)	Wald statistic	β-coefficient (95% CI)	Wald statistic
Spirometry indices					
FVC (L)	Ozone only	−0.03 (−0.05, −0.01)*	7.25*	−0.02 (−0.04, 0.003)	2.95
	Ozone + PM_{10}[a]	−0.03 (−0.05, −0.004)*	5.55*	−0.02 (−0.04, 0.01)	2.43
FEV_1 (L)	Ozone only	−0.01 (−0.03, 0.003)	2.65	−0.02 (−0.04, 0.01)	1.70
	Ozone + PM_{10}[a]	−0.01 (−0.03, 0.01)	0.70	−0.01 (−0.04, 0.01)	1.26
PEF (L/s)	Ozone only	0.004 (−0.07, 0.08)	0.01	−0.05 (−0.14, 0.04)	0.71
	Ozone + PM_{10}[a]	0.02 (−0.06, 0.10)	0.23	−0.05 (−0.14, 0.04)	0.62
$FEF_{25–75\%}$ (L/s)	Ozone only	0.01 (−0.04, 0.05)	0.07	0.03 (−0.04, 0.09)	0.39
	Ozone + PM_{10}[a]	0.02 (−0.02, 0.07)	1.05	0.03 (−0.04, 0.10)	0.41
Self measured peak expiratory flow (Mini-Wright)					
PEF[b] (L/min)	Ozone only	4.80 (1.17, 8.43)*	6.73*	3.45 (−1.14, 8.05)	2.17
	Ozone + PM_{10}[a]	5.34 (1.62, 9.06)*	8.08*	3.37 (−1.39, 8.13)	2.03
Exhaled nitric oxide fraction (F_{eNO}) log-transformed					
F_{eNO} (ppb)[c]	Ozone only	11.10 (4.23, 18.43)*	10.43*	11.79 (2.61, 21.80)*	6.49*
	Ozone + PM_{10}[a]	9.48 (2.46, 16.98)*	7.17*	11.77 (2.54, 21.82)*	6.41*

Note: Results of mixed models adjusting for sex, height, weight, exposure area (low/high), study area (Athens/Thessaloniki), years of father's education, air temperature (7-d average), mean time spent outdoors daily, citrus fruits consumption (yes/no), and week ID.
[a]7-d average, measurements from nearest fixed site.
[b]Weekly averages from daily values (daily value is the average of three maximum values of morning, noon, and night measurements).
[c]In this model we did not adjust for height, weight, and week ID.
*$p < 0.05\%$.

with PEF were observed, with the largest study (at Pine Springs Ranch, east of Los Angeles) reporting a positive and statistically significant association (Kinney et al. 1996). In a Brazilian study, Castro et al. (2009) observed a protective effect of O_3 to school children's PEF. Recently, Altuğ et al. (2014) showed that PEF levels were negatively associated with weekly average O_3 levels only in children without upper respiratory tract complaints.

Two panel studies examined the effect of O_3 exposure on FEV_1 in asthmatic children and found no effect (Dales et al. 2009; Delfino et al. 2004). Both of them relied on self-measured FEV_1 by handheld electronic devices. More consistent findings have been observed in the few studies using spirometry performed by trained personnel. In a reanalysis of six summer camp studies, a consistent decrease in FEV_1 has been found associated with increased ambient exposure to O_3 (Kinney et al, 1996). In the Mexican study (Barraza-Villarreal et al. 2008), using fixed site outdoor measurements, the investigators report negative associations with FVC and FEV_1, although none reached the nominal level of significance. In a study in Taiwan (Chang et al. 2012), based also on fixed site measurements for the assessment of O_3 exposure, a significant negative association was observed with FVC and a nonsignificant negative association with FEV_1. The decrements, we observed mainly in FVC and also in FEV_1, are also consistent with previous experimental studies in young adults. The main reason for this O_3-induced decrease is believed to be impaired inspiration as a result of stimulation of airway receptors causing a reduction in the level of inflation achieved at full inspiration (Blomberg et al. 1999; Hazucha et al. 1989; Kjærgaard et al. 2004). Based on all the above, our results support that ambient O_3 exposure affects inspiratory capacity and is related to airway inflammation.

In the present study, we also found a significant increase in respiratory symptoms after increased exposure to O_3. This is a consistent finding among studies that investigate O_3 effects at different time scales, mostly in asthmatic children. Thus, Declerq and Macquet (2000), Delfino et al. (2002), Escamilla-Nuñez et al. (2008), Just et al. (2002), Mortimer et al. (2002), and Schlink et al. (2002) found an association of O_3 exposure and increase in respiratory symptoms, but few others found no effect (Jalaludin et al. 2004; Ostro et al. 2001). In our study asthmatic children were not found to be more susceptible than nonasthmatic children and this has been reported by other studies as well (Ward et al. 2002).

Our study has certain limitations. Our sample included children who agreed to participate from randomly selected schools; however, they did not form a random sample from the students of their class. This may have introduced some selection bias because children from more educated families or families more sensitive to environmental problems may have been preferentially included. However, we think that this did not bias our results away from the null given that the students attending a specific school are socioeconomically comparable (they live in the neighborhood) and the percentage of asthmatic children in our sample is comparable to that reported for the population (Papadopoulou

Table 4. Association between the number of days with any respiratory symptom or absenteeism with weekly O_3 exposure measured with personal monitors in the panel of 178 school children.

Health outcome	Model	All study periods % change per 10 μg/m^3 O_3 (95% CI)	Wald statistic
Number of days with any symptom[a]	Ozone only	19.16 (−0.53, 42.75)	3.62
	Ozone + PM_{10}[b]	20.94 (0.42, 45.66)*	4.02*
Number of days of absenteeism[c]	Ozone only	−28.95 (−55.25, 12.79)	2.10
	Ozone + PM_{10}[b]	−31.32 (−56.88, 9.39)	2.50

Note: Results of Poisson models adjusting for sex, exposure area (low/high), study area (Athens/Thessaloniki), years of father's education, air temperature (7-d average), mean time spent outdoors daily, citrus fruits consumption (yes/no), and week ID.
[a]Number of days within the week that any symptom occurred.
[b]7-d average, measurements from nearest fixed site.
[c]Number of days within the week that the student was absent from school.
*$p < 0.05\%$.

et al. 2011). Another limitation of our project is the fact that assessment of exposure to pollutants other than O_3 was not based on personal measurements but was estimated using the nearest fixed site monitors. This might have led to residual confounding, especially taking into account exposure to PM. We believe that the probability of residual confounding is limited because the correlations of O_3 with PM_{10} and NO_2 was not the same across the high- and low-O_3 areas and between the two cities. Furthermore, PM is more homogeneously distributed and penetrates indoors making the fixed site and personal measurements less different from those of O_3. Absenteeism might be a source of bias to the null for the estimated effects. During the field work, on each of the days our researchers visited the schools for spirometry, only a few children were absent from school (0–4 children each time, except during the last summer week in Thessaloniki when 17 children could not be found because specific schools had end-of-year activities), and it may be hypothesized that some of these children might have had a respiratory problem because the reason for the absence was not reported by the school. Another limitation of our study is the inability to study children during the hottest months of the year, July and August, because of school holidays. These months are also characterized by high-O_3 concentrations. We were not able to reach schoolchildren during the holidays, but it should be mentioned that most families in Greece take their holidays during this time and the population of children in the cities is greatly reduced.

An advantage of the present study is the use of personal O_3 monitoring. The higher contrast observed in outdoor school measurements compared with personal ones denotes the importance of relying on personal measurements when assessing O_3 exposure health effects on school children. Another advantage is the spirometry performed by physicians, which provides more accurate and valid results compared with self-monitoring. This may explain our findings associating ozone exposure with the weekly FVC and FEV_1, provided through spirometry, whereas an analysis of daily PEF measured by peak flow meters, handled by the same sample of students, did not provide significant results (Samoli et al. 2017).

Among the strengths of our study is the study design, as panel studies allow great flexibility in investigating multiple exposures and multiple outcomes; the location, as Greece is a warm, sunny country with inhabitants exposed to high-O_3 concentrations; the large number of students and the relatively long period of follow up; the limited number of participants lost to follow-up or with incomplete data; the personal O_3 monitoring; and the wealth of data collected over a variety of respiratory morbidity indices using standardized procedures. Moreover, our results were robust after controlling for particles and under several sensitivity/subgroup (e.g., after considering only the asthmatic schoolchildren) analyses.

Conclusion

The study provides evidence that airway inflammation and the frequency of respiratory symptoms increase while lung function decreases with increased O_3 exposure in school children generally representative of the population of children 10- to 11-y-old in two large Southern European cities, where high- ozone concentrations are observed. Schoolchildren should be considered at a moderate risk of suffering respiratory health adverse effects following short-term exposure to the current levels of ambient O_3, in line with what has been found in controlled human exposure studies at ambient concentrations. Focusing on asthmatic children may lead to an underestimation of the real burden of O_3 induced respiratory adverse events.

Acknowledgments

The work was cofunded by the European Commission and the Greek government through the National Strategic Reference Framework 2007–2013 contract ref RESPOZE-children/2248.

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